ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 1985, p. 239–245
0066-4804/85/020239-07$02.00/0

Vol. 27, No. 2

Treponemycin, a Nitrile Antibiotic Active Against *Treponema hyodysenteriae*

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Received 8 July 1984/Accepted 20 November 1984

Two strains of *Streptomyces* sp. (isolates 275 and 124) were isolated from soil samples collected from the fields around Lewiston, Idaho. Based on their cellular morphology and physiology, the two isolates were identified as *Streptomyces albovinaceous*. Both isolates produced an antibiotic when grown in liquid culture medium containing homogenized oats. The antibiotic (treponemycin) was isolated from the culture broth by solvent extraction and purified by silica gel column and preparative thin-layer chromatographies. Treponemycin is a crystalline light-yellow compound with an mp of 93 to 95°C, levorotatory, and soluble in most organic solvents. It is sparingly soluble in water but insoluble in petroleum ether. On the basis of elemental analysis and mass spectral data, the molecular formula of the antibiotic was deduced to be $C_{28}H_{43}O_6N$. The infrared spectrum of the antibiotic indicated the presence of unsaturation, nitrile, lactone, or ester or all three functions, and carbonyl functions in the molecule. A sharp infrared absorption band for nitrile at 2,220 cm^{-1} and the presence of an unsaturated group indicated that the nitrile function may be attached to an unsaturated carbon atom. The presence of dienenitrile functions was further supported by the UV absorption spectrum of the antibiotic, which gave a UV_{max} at 257 nm. The proton magnetic resonance spectrum of the antibiotic did not give any peak which could be exchanged with deuterated water, which is an indication of the absence of carboxylic and hydroxyl functions in the molecule. All of the functional groupings indicated by the infrared and UV spectra of the molecule were further confirmed by the ^{13}C-magnetic resonance spectrum of the compound. A brief hydrogenation of the antibiotic yielded a biologically active tetrahydro derivative, whereas extended hydrogenation produced an inactive primary amine. Mild alkaline hydrolysis and subsequent esterification of the antibiotic with diazomethane produced an inactive dimethyl ester. Apparently both the nitrile and the lactone functions are essential for the treponomycin molecule to show antimicrobial activity. The antibiotic showed inhibitory activity against several species of bacteria, especially *Treponema hyodysenteriae*, the causative agent of swine dysentery. In view of the oral 50% lethal dose of 400 mg/kg and its low MIC against four strains of *T. hyodysenteriae*, the antibiotic may have value as a swine dysentery therapeutic.

In the course of our screening program to find new and active compounds from soil microorganisms, we isolated two cultures of *Streptomyces* sp. (isolates 124 and 275), both of which we later identified as *Streptomyces albovinaceous*. Both of these cultures produce an active substance which showed a strong inhibitory activity in vitro against four pathogenic strains of *Treponema hyodysenteriae*, the causative agent of swine dysentery. This report describes the characteristics of the antibiotic-producing organism and the isolation, purification, and characterization of the antibiotic produced by it.

MATERIALS AND METHODS

Organism. The organism was isolated from soil samples collected from fields in the vicinity of Lewiston, Idaho. *S. albovinaceous* can be maintained on potato dextrose agar, oatmeal agar (OMA), and glycerol-peptone-asparagine agar slants or plates or both. For long-time storage the organism was transferred to sterile soil and kept at room temperature. In sterile soil the organism remained viable for more than 6 months.

Streptomyces sp. isolates 124 and 275 were characterized by the methods developed by collaborators of the International Streptomyces Project for the identification of *Streptomyces* species (1, 7, 8, 10–14). Identification was made by using Nonomura's classification key (7) and *Bergey's Manual of Determinative Bacteriology* (1). The color of mature aerial mycelia was observed on OMA, potato dextrose agar, and Pridham and Gottlieb's basal agar with glucose (8). The colony color on the reverse side of the plate was observed on potato dextrose agar, OMA, and tyrosine-casein-nitrate agar. Color standards and color nomenclature were used to describe the colony color. The production of melanoid pigments was examined on peptone-iron agar, tyrosine agar, and tryptone-yeast extract broth. Carbon utilization by the organism was studied on Pridham and Gottlieb's basal agar with various carbon sources added (8).

The morphologies of sporophores, spores, and spore surfaces were determined with a scanning electron microscope. For this study the organism was cultured on OMA for 1 week at 30°C. The material was fixed in 3% glutaraldehyde, stained in 2% osmium tetroxide solution, and dehydrated through a graded ethanol series. Material was then subjected to critical-point drying and sputter coated with gold.

Production of the antibiotic. Cultures of *Streptomyces* sp. isolates 124 and 275, which were grown on glucose-peptone agar (in grams per liter of distilled water: glucose, 10.0; peptone, 1.0; $MgSO_4 \cdot 7H_2O$, 0.5; K_2HPO_4, 0.5; asparagine, 0.5; agar, 20.0), were used to inoculate 500-ml Erlenmeyer flasks containing 200 ml of sterile oatmeal broth at pH 6.5. Rolled oats were homogenized to a fine powder in a Waring blender before addition to the culture media. For preparation of the oatmeal broth a 20-g quantity of powdered oats was suspended in 1 liter of tap water and sterilized for 35

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min. No additional carbon, nitrogen, or mineral sources were added. The seed culture was grown in this medium for 4 days at 28°C on a reciprocating shaker set at 250 rpm. A fermentor jar containing 10 liters of the aforementioned sterile oatmeal broth was inoculated with the seed culture, allowed to grow aerobically for 6 days at 28°C with 2 liters of sterile air per min, and agitated at 300 rpm.

Antimicrobial assays and mammalian toxicity. The antimicrobial activities of the fermentation broth, the crude solvent extract of the broth, and the purified antibiotic samples were tested by using the paper disk agar diffusion bioassay method against *Bacillus subtilis* and *Saccharomyces cerevisiae* as the test organisms.

The MICs of treponemycin on several species of bacteria and fungi were determined by the agar diffusion method. The MICs against four strains of *T. hyodysenteriae* were determined in vitro by incorporating the antibiotic into a blood agar similar to that described by Songer et al. (15). The strains of *T. hyodysenteriae* used for inoculation were cultivated in a liquid medium similar to that described by Kinyon and Harris (4). Bottled culture flasks inside GasPak jars (BBL Microbiology Systems) were shaken during incubation at 37°C for 2 to 3 days. The number of cells used was standardized microscopically to approximately 10^6, 0.1 ml of which was used for inoculation.

The 50% lethal dose of the antibiotic on mice was determined after oral administration of the antibiotic.

Isolation and purification. After 6 days of growth the whole broth was extracted twice, each time with 3 liters of methylene chloride. As an alternate procedure the active substance can be extracted with other water-immiscible organic solvents such as ethyl acetate, chloroform, butanol, and diethyl ether. Methylene chloride is the preferred solvent since it is less volatile, does not form troublesome emulsions, and also is nonpolar enough to leave behind some polar impurities. Both fractions of methylene chloride extracts were combined and concentrated under reduced pressure to a viscous yellow liquid. The crude material (2 to 3 g) was dissolved in chloroform (10 ml) and adsorbed on a dry Silica Gel G column (2.5 by 30 cm packed Silica Gel G60; particle size, 0.04 of 0.063 mm; E. Merck AG). The column was washed successively with 1 liter each of petroleum ether, methylene chloride, and chloroform, which removed most of the pigments and nonpolar impurities. The active material along with some impurities was eluted with a mixture of chloroform-acetone (9:1, vol/vol). After removal of the solvent, the active fraction was applied to silica gel preparative thin-layer chromatographic (TLC) plates (Baker Si 250F) and developed with an ethyl acetate-acetone mixture (2:1, vol/vol). On the chromatogram under UV light, the active compound appeared as a dark quenching band that was scraped off the TLC plate and extracted with acetone. The antibiotic was crystallized from chloroform-hexane as light-yellow crystals (450 mg).

The homogeneity of the antibiotic was determined in different solvent systems, using TLC. On these chromatograms the antibiotic was visualized under UV light and also by the Libermann reagent (concentrated H_2SO_4-ethanol, 11:9). The homogeneity of the antibiotic was also checked by high-pressure liquid chromatography techniques with a reverse-phase C_{18} column (Aquapore RP-300, 10 μm), using acetonitrile and trifluoroacetic acid-water (0.1% trifluoroacetic acid in water) as eluting solvents. The antibiotic was detected in the column effluents at 254 nm.

Spectroscopic procedures. UV and visible spectra of the antibiotic were measured in spectral-grade methanol on a Gilford 3600 spectrophotometer. Infrared (IR) spectra of the antibiotic and its chemical derivatives were taken in chloroform on a Beckman spectrophotometer, model Acculab 1. Proton magnetic resonance (PMR) and ^{13}C-magnetic resonance spectra of the antibiotic and its derivatives were recorded on a Nicolet magnetic resonance spectrometer, model NT-20WB, at 200.042 MHz in deuterated chloroform, using tetramethylsilane as an internal reference. The mass spectrum of the antibiotic was taken with a Hewlett-Packard 598 gas chromatograph-mass spectrometer.

The specific rotation of the antibiotic was measured in chloroform solution at the 546-nm line of a mercury lamp with a Jasco-Dip-181 polarimeter.

Elemental analysis. Elemental analysis was performed in duplicate by Galbraith Laboratories.

Hydrogenation of the antibiotic. The antibiotic (70 mg) was dissolved in 5 ml of 95% ethanol in a 25-ml round-bottom flask to which a 10% palladium-on-charcoal catalyst (6 mg) was added. The hydrogenation was carried out at ambient temperature (20°C) under 1 atm of hydrogen pressure for 90 min. At the end of 90 min the contents of the flasks were filtered and washed twice with 95% ethanol and dried under reduced pressure. The resultant residue was dissolved in 1 ml of chloroform, applied on a silica gel column, and eluted with a solvent mixture of chloroform-ethyl acetate (2:1). The hydrogenated product was crystallized from methylene chloride-hexane as a white crystalline product (60 mg).

In another experiment hydrogenation was carried out for 180 min. A 40-mg sample of the antibiotic was dissolved in 4 ml of absolute ethanol in a 25-ml round-bottom flask to which 3 mg of a 10% palladium-on-charcoal catalyst was added. Hydrogenation under 1 atm of pressure of hydrogen gas was carried out for 180 min. At the end of hydrogenation, the contents were filtered and the solvent was evaporated to dryness. The residue was crystallized in a methylene chloride-hexane mixture (36 mg).

Esterification of the antibiotic. A 50-mg sample of the antibiotic was dissolved in 5 ml of methanolic potassium hydroxide (1 N), with stirring at room temperature for 16 h. The reaction mixture was diluted with water (60 ml), acidified with 2 N HCl, and extracted twice with 50 ml of diethyl ether. The ether extract was treated with diazomethane, which was generated from alcoholic KOH and Diazatid (Aldrich Chemical Co.). At the end of the reaction the excess diazomethane was allowed to escape at room temperature or until the liquid was colorless. The solution was dried under vacuum, and the resulting material was found to be homogeneous by TLC.

RESULTS

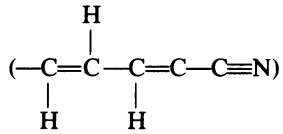
Characteristics of the organism. The morphological and physiological characteristics of the organism are summarized in Table 1. By using the International Streptomyces Project classification scheme and *Bergey's Manual of Determinative Bacteriology* (1), the organism can be classified readily under *Actinomycetales*, family *Streptomycetaceae*, genus *Streptomyces*, and species *albovinaceous*. The organism utilized all carbon sources tested except inositol and raffinose (Table 1). In liquid culture it produced light wine red-colored pigment, but it was negative for melanin production. *S. albovinaceous* produced coenocytic aerial mycelia with abundant oidiospores on OMA, potato dextrose agar, and other microbiological media that are commonly used to grow actinomycetes. The spores were cylindrical (0.7 by 1.2 μm) and smooth walled (Fig. 1) and were formed on flexuous sporophores.

TABLE 1. Morphological and physiological characteristics of *Streptomyces* sp. isolates 275 and 124

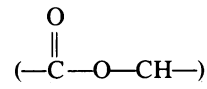
Characteristics tested	Result
Aerial mass color	White
Melanoid pigment production	None
Reverse-side pigment	Not produced
Soluble pigment	Not produced
Spore chain morphology	Sporophores, flexuous (10 to 20 spores per sporophere)
Spore wall ornamentation or spore surface	Smooth-walled oidiospores (cylindrical, measuring 1.2 by 0.8 μm)
Carbon utilization[a]	
Arabinose	+
Xylose	+
Inositol	−
Mannitol	+
Fructose	+
Rhamnose	+
Sucrose	+
Raffinose	−
Starch	+
Glucose	+
Galactose	+
Salicin	+
Antibiotic production	Produces dienenitrile antibiotic
Streptomycin susceptibility	Inhibited at 50 μg/ml, but not at 10 μg/ml
Identification	*Streptomyces albovinaceous*

[a] +, Carbon utilized; −, carbon not utilized.

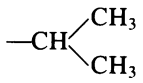
Physical, chemical, and biological properties of the antibiotic. (i) The parent compound. The antibiotic as purified was homogeneous by both TLC and high-pressure liquid chromatography. Treponemycin is a light-yellow-colored substance with an mp of 93 to 93.5°C and a specific rotation, $[\alpha]_{546}^{21}$, of −17.47 (C, 4.3; $CHCl_3$). It is soluble in most organic solvents except ether, hexane, and carbon tetrachloride. The mass spectrum furnished a molecular ion peak at *m/e* 489 (Fig. 2A), which is consistent with a molecular formula of $C_{28}H_{43}O_6N$ (found: $C_{68.82}$, $H_{9.13}$, $N_{2.84}$, $O_{19.21}$ by difference; calculated: $C_{69.32}$, $H_{8.76}$, $O_{19.12}$, $N_{2.79}$). The IR spectrum (Fig. 3A) of the antibiotic in chloroform showed absorptions at 3,000 (olefinic C—H stretching), 2,950, 2,910, 2,220 (nitrile stretching), 1,720 (lactone or ester), 1,700 (carbonyl stretching), 1,635 (C=C stretching), 1,460, 1,375, 1,250, 1,170, 1,025, and 965 cm^{-1} (substituted olefinic). The presence of an olefinic group and a sharp band for a nitrile function at 2,220 cm^{-1} suggested that the nitrile group may be attached to an unsaturated carbon. Furthermore, the UV spectrum of the compound in methanol gave UV maxima at 257 nm, which confirms the presence of a conjugated dienenitrile group in the molecule:



The PMR spectrum (Fig. 4A) of the antibiotic in deuterochloroform, using tetramethylsilane as internal standard, indicated the presence of 43 protons, 3 of which were attached to the conjugated olefinic system. A downfield doublet at δ6.81 (1H, J = 11 cps) was assigned to the proton adjacent to the nitrile function. There were two multiplets at 6.40 (1H). The downfield doublet at δ4.97 (1H, J = 11 cps) suggested that the lactone or ester function of the antibiotic terminates on a secondary carbon atom



Four quarternary methyl groups absorb at δ0.79, 0.82, 0.85, and 1.25. The downfield singlet at δ1.25 suggests that three protons are attached very near to the carbonyl ester or olefinic function. The doublet for six protons at δ1.043 (J = 6 cps) suggests the presence of a



system in the carboxylic acid proton or hydroxyl proton since none of the protons was exchanged with D_2O.

The ^{13}C-magnetic resonance of the antibiotic showed absorption lines at δ207.74, 177.65, and 172.25. This suggested that the antibiotic must have at least one carbonyl and two lactone or ester groups, which altogether accounted for five oxygen atoms. Since there was no D_2O-exchangable proton in the PMR spectrum and no absorption band for hydroxyl groups in the IR spectrum, we deduced that the remaining oxygen atom in the molecule might be present as an ether (—C—O—C—) linkage. This inference was confirmed from the ^{13}C-magnetic resonance spectrum of the antibiotic, which had four absorption lines, two for lactone or ester and two for ether, in the region of δ80–70, which is the characteristic absorption range for carbons bonded singly with oxygen. The presence of four olefinic and one nitrile carbon was also confirmed from the ^{13}C-magnetic resonance spectrum, which exhibited five absorption lines in the region δ119.0 to 144.0.

The MICs of treponemycin for selected bacteria are presented in Table 2. The antibiotic is active against mainly gram-positive and a few gram-negative species of bacteria,

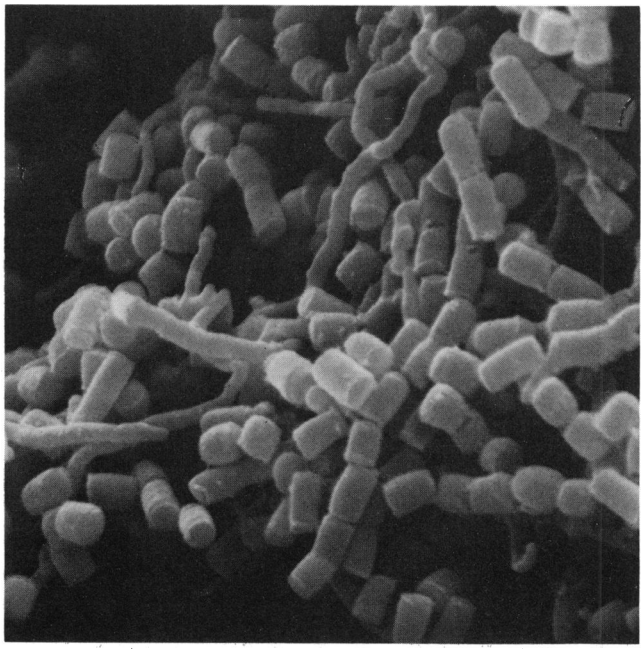


FIG. 1. Scanning electron micrographs of *S. albovinaceous* showing vegetative mycelia and mature spores.

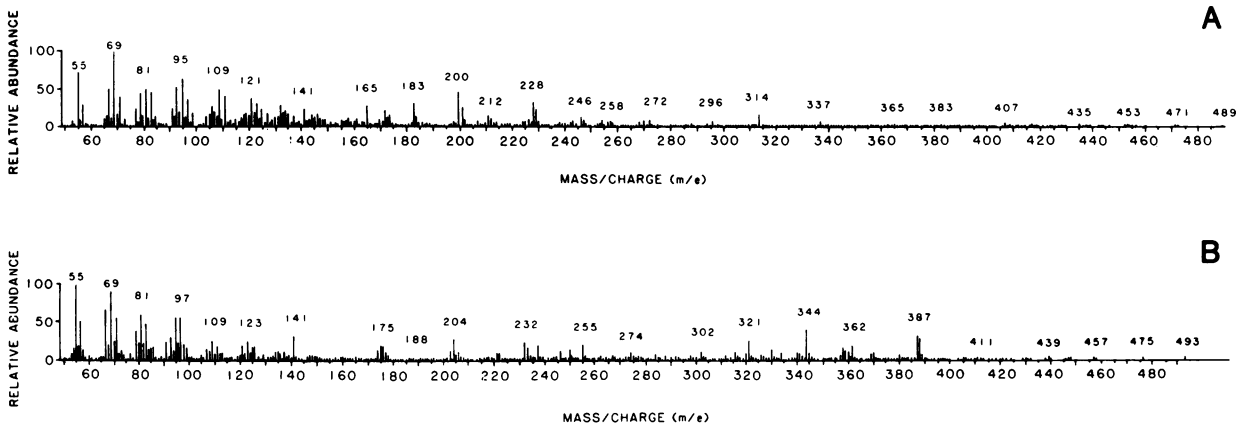


FIG. 2. Mass fragmentation patterns of (A) treponemycin and (B) tetrahydrotreponemycin.

bacteriodes, and actinomyces. When tested, treponemycin showed no activity against *Candida albicans* and *Aspergillus niger*. However, the antibiotic exhibited strong activity in vitro against the organisms associated with swine dysentery. Four strains of *T. hyodysenteriae* (Table 2) which were previously isolated from characteristic cases of swine dysentery and were also known to produce typical pathological symptoms in the mouse model (J. W. Whalen et al., presented at the Michigan Branch-Western Ontario Branch joint meeting, American Society for Microbiology, 1982) were inhibited at or below 5 μg of the antibiotic per ml of test medium.

The 50% lethal dose (mouse) of the antibiotic was slightly less than 400 mg/kg of body weight.

(ii) Tetrahydro derivative of the antibiotic. A brief 90-min hydrogenation of the parent molecule resulted in the formation of a major white crystalline product. This was purified by TLC and later found to be homogenous by TLC plate assay (methylene chloride-methanol, 9:1, R_f 0.58). The IR and PMR spectra of the product showed no characteristic absorptions for unsaturation; however, there was a peak at 2,220 cm^{-1} in the IR spectrum. Moreover, an increase of four mass units in the molecular ion (M^+493) as well as major fragments above *m/e* 383 suggested that the compound was the tetrahydro derivative of the parent antibiotic (Fig. 2B). The antimicrobial activity of the tetrahydro derivative remained unchanged when compared with that of the parent antibiotic.

(iii) Primary amine of the antibiotic. An extended 180-min hydrogenation of the antibiotic resulted in formation of another white crystalline compound with a specific rotation, $[\alpha]_{546}^{21}$, of −10.154 (C, 3.25; $CHCl_3$). The IR spectrum of this product revealed the absence of bands at 2,220 (nitrile stretching), 1,635 (C=C stretching), and 965 cm^{-1} (substituted olefin) and the presence of two peaks in the region of 3,300 to 3,500 cm^{-1} for a primary amine (N—H stretching) (Fig. 3C). The primary amine of the antibiotic was biologically inactive.

(iv) Dimethyl ester of the antibiotic. Esterification of the antibiotic resulted in formation of a dimethyl ester. IR spectra (Fig. 3B) showed bands at 3,520 (OH stretching) and 1,730 (lactone or ester carbonyl) cm^{-1} and a sharp band at 1,260 cm^{-1} (C—O stretching). Morover, the PMR spectrum of the product furnished a sharp singlet for six protons at δ3.74, indicating the presence of two methyl esters in the molecule and two singlets at δ3.32 (1H) and 3.3 (1H) for hydroxyl protons. The dimethyl ester of the antibiotic was biologically inactive.

DISCUSSION

Both soil isolates of *Streptomyces* sp. that produce treponemycin were identified as *S. albovinaceous*. It has been reported that the same species of *Streptomyces* also produces another antibiotic (Nancimycin), which is active against gram-positive bacteria and certain viruses (R. Sonvick, J. F. Pagano, B. Brook, and J. Vandeputte, U.S. patent 2,999,048, 1961). The chemical and other properties of Nancimycin seem to be distinct from those of treponemycin. For example, the three UV absorption bands of Nancimycin (at 225, 305, and 425 μm) are absent in the UV spectra of treponemycin. Unlike treponemycin, the antibiotic Nancimycin is a non-nitrogenous compound with a molecular formula of $C_{23}H_{30}O_{11}$ (treponemycin, $C_{28}H_{43}O_6N$). In addition to these differences, the characteristic IR absorption band at 2,210 nm for the nitrile function of treponemycin is absent in the IR spectra of Nancimycin. These characteristics indicate that Nancimycin and treponemycin, although produced by the same species of *Streptomyces*, are actually two different and distinct compounds. The production of structurally different antimicrobial compounds by common species of *Streptomyces* is not an uncommon phenomenon, since there are abundant instances in the literature in which the same species of *Streptomyces* is known to produce more than one group of antibiotics (1, 16).

Assignment of nitrile, diene, and other functional groupings in the molecule of treponemycin is based on spectroscopic and chemical analyses of the antibiotic and its derivatives. A sharp absorption band at 2,220 cm^{-1} in the IR spectrum followed by an absorption line at 144.00 in the ^{13}C-magnetic resonance spectrum indicate the presence of a nitrile group in the molecule. Furthermore, the suggested molecular formula of $C_{28}H_{43}O_6N$ and a yield of 1 mol of ammonia per mol of the compound upon acid hydrolysis not only indicate the presence of a nitrile group but also confirm the presence of only one nitrile group in the molecule.

Although a nitrile or isonitrile functional group is rarely found in natural products (2, 6), recently a group of isonitrile-containing compounds was isolated from several species of the soil-inhabiting fungus *Trichoderma* (3). In addition, an inhibitor (A32390A) of dopamine β-hydroxylase, which is also an antimicrobial compound, was isolated and

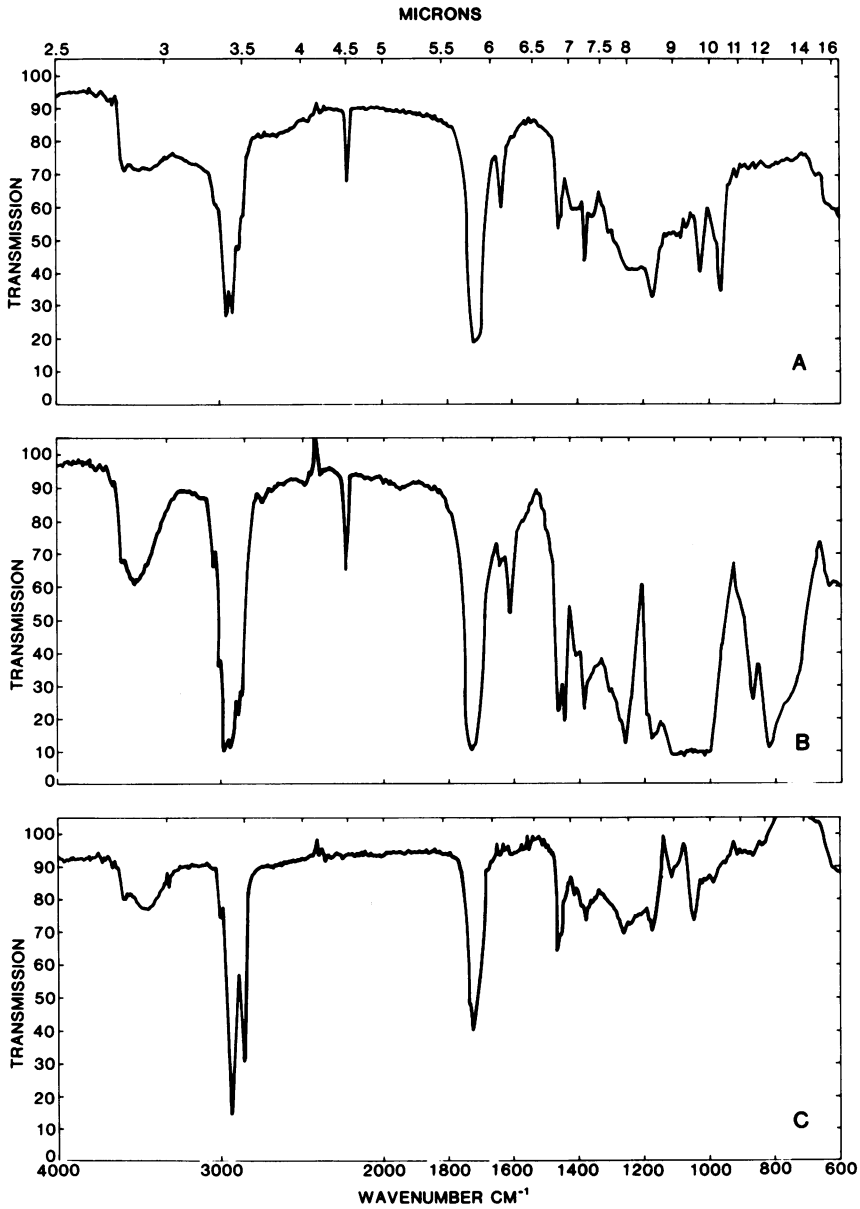
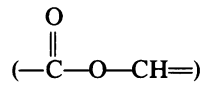


FIG. 3. IR spectra of (A) treponemycin; (B) treponemycin, dimethyl ester; and (C) treponemycin, amine in chloroform.

characterized (5). The chemical, physical, and biological properties of these compounds appear to be different from those of treponemycin (2, 3, 5, 6).

Assignment of a diene function in the molecule was also made on the basis of PMR and ^{13}C-magnetic resonance spectra of treponemycin; the spectra further indicate that the molecule may contain three olefinic protons and four unsaturated carbons in addition to nitrile carbon. The UV spectrum of the compound in methanol furnished an absorption maximum at 257 nm, which is a strong indication of a nitrile group, possibly conjugated with a diene system, since saturated nitriles are transparent in the near-UV region (9) and the α,β-unsaturated nitriles absorb at 257 nm. Hence all of these observations strongly suggest that the three olefinic protons of the diene system are in conjugation with that of a nitrile group. In addition, a doublet at δ6.81 for one proton (J = 11 cps) was assigned for the hydrogen attached to α-carbon with respect to nitrile, which is *trans* to the hydrogen (δ6.40) at β-carbon. The remaining olefinic proton appeared at δ4.97 (1H, J = 11 cps) and suggested that the lactone or ester function in the molecule terminates on a secondary carbon



Absence of any D_2O-exchangable protons in the molecule clearly excludes the presence of hydroxyl or carboxyl groupings in the molecule. In addition, there is no evidence of loss of 44 mass units for CO_2 from molecular ion or fragment ions of high mass of the parent compound or its tetrahydro derivative; this substantiates the absence of a carboxylic group in the molecule.

The assignment of carboxyl, lactone, or ester and ether groupings in the molecule was based on IR and ^{13}C-magnetic resonance spectra of the antibiotic. Absorption lines were

assigned at δ207.74 for carboxyl and at δ177.65 and 172.25 for lactone or ester; of four absorption lines in the region of δ 80–70 of the ^{13}C-magnetic resonance spectra, two were assigned for lactone or ester and two were assigned for ether. These observations were further supported by the IR spectrum (Fig. 3A).

Brief hydrogenation of the molecule resulted in complete reduction of the diene part of the molecule, but the nitrile function of the compound remained intact, as shown by the presence of IR absorption bands at 2,220 cm^{-1}. Bioassay of this product showed no loss in antimicrobial activity when compared with the parent compound. The longer hydrogenation of the antibiotic yielded a primary amine which showed no antimicrobial activity. In addition, hydrolysis and esterification of the antibiotic resulted in formation of a dimethyl ester which again showed no antimicrobial activity. In the dimethyl ester of the antibiotic molecule, opening of the lactone ring possibly resulted in total loss of biological activity, even though the diene and nitrile functions were intact. This suggests that the stereospecificity of the molecule is essential for the antimicrobial property. It also suggests that both nitrile and lactone functions are needed for this molecule to exhibit antimicrobial activity.

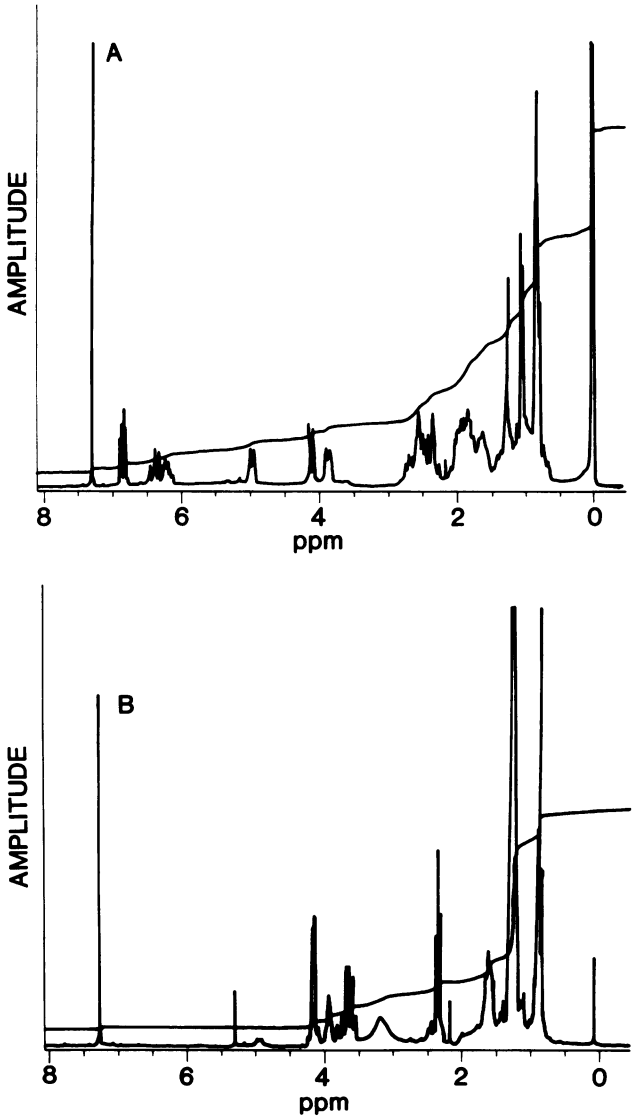


FIG. 4. PMR spectra of (A) treponemycin and (B) treponemycin, amine in deuterochloroform.

TABLE 2. Antibiotic spectrum of treponemycin

Microorganism	MIC (μg/ml)
Actinomyces viscosis	<0.5
Bacillus subtilis	1.0
Bacteriodes fragilis	5.0
B. multiacidus	>100.0
Clostridium perfringens	>100.0
C. septicum	>100.0
Erwinia amylovora	50.0
Escherichia coli	>100.0
Fusobacterium necrophorum	<0.1
Lactobacillus acidophilus	50.0
Pseudomonas aeruginosa	>100.0
Salmonella typhimurium	>100.0
Sarcina lutea	<0.1
Staphylococcus aureus	100.0
Streptococcus faecalis	25.0
S. mutans	50.0
S. bovis	>100.0
Treponema hyodysenteriae strain	>2.5 < 5.0
B-78	>2.5 < 5.0
B-140	>2.5 < 5.0
B169	>2.5 < 5.0
T-9-J	>2.5 < 5.0

The MIC on several species of bacteria indicates that, although treponemycin exhibits a broad spectrum of antimicrobial activity, the activity against several species of bacteria is too weak to be of any practical importance. Nevertheless, the antibiotic also showed good activity in vitro against four pathogenic strains of *T. hyodysenteria*, the causal agents of swine dysentery, a disease of considerable economic importance in the swine industry. In view of its low mammalian toxicity (50% lethal dose of slightly less than 400 mg/kg) and its strong activity against *T. hyodysenteriae*, the antibiotic may have value as a swine dysentery therapeutic.

Since treponemycin showed strong activity against one species of *Treponema*, it is worthwhile to explore the efficacy of this compound not only on other pathogenic species of *Treponema* such as *T. pallidum* (the causative agent of syphilis), *T. pertenue* (the causative agents of yaws), *T. carateum* (the causative agent of pinta), and *T. paraleuis-cuniculi* (the causative agent of rabbit syphilis), but also on other genera of spirochetes that are the causative agents of various diseases in humans and other domestic animals.

ACKNOWLEDGMENTS

We thank Rosy Chaco for assistance in obtaining scanning electron micrographs and Sharon Truitt and R. W. Brosemer for critical review of this manuscript. We also thank Judy Okita for assistance in manuscript preparation.

This study was funded in part by the Dow Chemical Co., U.S.A., Midland, Mich., and by the Graduate School of Washington State University.

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